

*I. Rejection Under 35 USC §112, First Paragraph*

The examiner has rejected claims 1-4, 6-9, 11, 12 and 14 under 35 USC §112, first paragraph, on the basis that

[T]he disclosure is enabling only for claims limited to transgenic mammals and methods of making transgenic non-human mammals selected from the group consisting of mice, rats, rabbits, pigs, sheep and goats which produce biologically active protein C in their milk and methods of producing biologically active protein C selected from the group consisting of mice, rats, rabbits, pigs, sheep and goats.

Office action at page 2. Accordingly, applicants understand the examiner's position to be that the specification does not support (1) the production of transgenic cows, (2) the production of a protein in a transgenic mammal with the exception of protein C, and (3) the production of a form of protein C that is not biologically active. Applicants respectfully traverse this basis for rejection.

With regard to the first basis for rejection, the examiner has stated that:

Applicant's species claim to transgenic cows is not enabled by the disclosure, nor are there sufficient teachings in the art for the production of a transgenic cow that the specification does not have to provide guidance for this species.

Office action at page 2. Applicants respectfully submit, however, that the disclosure of the specification does provide sufficient guidance for production of transgenic cows.

In particular, the present application describes the generation of transgenic mice and pigs by microinjection of a DNA construct into pronuclei of zygotes, using the methods described by Hogan et al., MANIPULATING THE MOUSE EMBRYO (Cold Spring Harbor Press 1986). See the specification at page 27, second full paragraph, and at page 28, first full paragraph. Krimpenfort et al. also used the microinjection technique of the Hogan reference to produce transgenic dairy cattle that contain the human lactoferrin gene. See page 847 (first full paragraph)

and reference 15 in Krimpenfort et al., *Bio/Technology* 9: 844-847 (1991) [Exhibit 1]. Thus, Krimpenfort et al. used microinjection methodology that was available at the time that the parent of the present application was filed to generate transgenic cattle that contain the coding sequence of a human gene.

Applicants also respectfully direct the examiner's attention to the 1994 review article by Brem et al., in which the authors state that:

At present, direct microinjection of DNA into the pronuclei of zygotes is the method of choice for the generation of transgenic livestock.

Page 182, first full paragraph, in Brem et al., "Large Transgenic Mammals," in *ANIMALS WITH NOVEL GENES*, (MacLean, ed.), pages 179-244 (Cambridge University Press 1994) [Exhibit 2]. Brem et al. also note that the "protocols for the preparation of DNA microinjection solutions used for farm animals are identical to those developed for mice (Hogan et al. 1986)." *Id.* at page 187, first full sentence. Moreover, Brem et al. review studies in which researchers produced transgenic cattle by microinjection. See the Brem publication at pages 221-229. Thus, Brem et al. provide evidence that the methods disclosed in the present specification can be used to generate transgenic cattle.

Nevertheless, the examiner has cited page 8 (column 5, paragraph 3) of Rudolph, "Advances Continue in Production of Proteins in Transgenic Animal Milk," *Genetic Engineering News* 15: 8 (October 15, 1995), as evidence that "there is nothing routine about making transgenic cows" because Rudolph reported that one company's work with transgenic cows has been suspended. Office action at page 3. The indicated paragraph, however, states that:

Not all companies have been able to parlay research results into milestone payments. **Transgenic Systems, Inc.** (Bozeman MT), for example, has suspended its efforts to express cytokines and antibodies in transgenic rabbits and cows. Having established ranching and agricultural arrangements, founder and president Kenneth DeBoer now is seeking funding and strategic partners so that his company can "fill a

niche that still exists for transgenic systems in large animals."

(original emphasis). That is, the Rudolph article indicates that Transgenic Systems, Inc. suspended its work on transgenic cows because the company is seeking new funding, and not because the company decided that the production of transgenic cows requires extraordinary experimentation. To the contrary, DeBoer stated that his company *will continue* transgenic work in "large animals" as soon as he obtains the funding. Thus, the paragraph does not evidence a general lack of enablement for producing transgenic cows.

The examiner also has referred to the Table in the Rudolph article that lists transgenic cows, among other transgenic animals, as being "under development." Applicants respectfully submit that the observation that work with transgenic cows is ongoing does not mean that one cannot obtain transgenic cows with routine experimentation. Moreover, applicants note that Pharming Health Care Products (PHP) have produced a *second generation* of transgenic cows that express human lactoferrin in milk. See the Rudolph article at page 8 (column 2, second full paragraph) and the entry for "lactoferrin" in the accompanying table. Rudolph also reports that the "core technology most often used" to generate transgenic animals is microinjection of the transgene into pronuclei of zygotes, which is the method disclosed in the present application. *Id.* at page 8, column four, last paragraph.

In sum, the attached publications and the Rudolph article provide abundant evidence that "[m]ethods for microinjection of other animal species are similar to the methods set forth" in the present specification for mice and pigs. Specification at page 29, first full paragraph. In particular, the publications evidence that the recitation of "cows" in the Markush group of transgenic animals complies with the requirements of §112, first paragraph.

The examiner's second basis for rejection is that "applicant has not taught a use for the production of inactive protein C."

Office action at page 3. Applicants understand the examiner's position to be that the claims should recite "biologically active protein C." Protein C, however, is normally produced as a zymogen which is subsequently proteolytically activated, and in this sense, the product expressed by the Protein C gene is "inactive" in nature. Significantly, the transgenic animals described in the specification produced an inactive form of protein C that applicants activated by proteolysis. See page 36, lines 10-14 of the present specification. Thus, the present claims comply with the first paragraph of §112.

The examiner's third basis for rejection is that "evidence is not of record that the method disclosed would produce any and all proteins in the milk of such a vast array of mammals." Office action at page 3. Applicants understand that the examiner's position to be that the claims should be limited to subject matter relating to the production of Protein C as the transgene.

Applicants note, however, that they have submitted data demonstrating the production of transgenic mice carrying an  $\alpha_1$ -antitrypsin gene under the control of the "long" (i.e., 4.2/4.1 kb) WAP promoter. See Exhibit 6 of the response filed on January 29, 1996. These transgenic mice secreted  $\alpha_1$ -antitrypsin into milk. *Id.*

In addition, applicants submit a copy of a manuscript by Paleyanda et al., entitled "Secretion of Human Furin into Mouse Milk." See Exhibit 3. This report presents data on the production of transgenic mice that carry a WAP promoter/human furin construct and a WAP promoter/human Protein C construct. Both constructs contained the long WAP promoter described in the present specification. See page 3, third full sentence, of the Paleyanda manuscript. Significantly, the transgenic mice secreted active human furin into the milk.

In sum, applicants have demonstrated the successful production of transgenic animals that express Protein C,  $\alpha_1$ -antitrypsin and human furin under the control of the long WAP

U.S. Serial No. 07/943,246

promoter. Applicants respectfully assert that this evidence is sufficient to support claims 12 and 14 under §112, first paragraph.

In light of the remarks above, applicants request the examiner to withdraw the rejection of the claims under 35 USC §112, first paragraph. Reconsideration of the claims is respectfully requested.

**CONCLUSION**

Applicants request reconsideration of the claims on their merits and respectfully solicit early notification of an allowance. If Examiner Crouch should have any questions or believes a telephone discussion would expedite prosecution, the examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

July 24, 1996  
Date

Phillip B.C. Jones  
Phillip B.C. Jones  
Registration. No. 38,195

FOLEY & LARDNER  
3000 K St., N.W., Suite 500  
Washington, DC 20007-5109  
(202) 672-5300